PATENT

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Signature

Appl No.	: 10/478,174	Confirmation No. 1948
Applicant	: Michael Täger, et al.	
Filed	: November 18, 2003	
Title	: Medicament Containing an Effector of the Glutathione Metabolism Together With Alpha-Lipoic Acid For Treating Diabetes Mellitus	
TC/A.U.	:	
Examiner	: Taofiq A. Solola	
Docket No.	: 1-16401	

DECLARATION OF MICHAEL TÄGER UNDER 37 CFR §1.132

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Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

Commissioner:

I, Michael Täger, hereby declare that:

1. I am named inventor of the invention disclosed and claimed in the subject patent application, U.S. Patent Application Serial No. 10/478,174. I received a PhD degree in Medicine (German Dr. med) from Otto-von-Guericke University, Magdeburg, in Germany in 1994. I hold a CEO position at IMTM GmbH, Germany. My responsibilities include scientific and financial management and I consider myself an expert in the area of study relating to the instant invention.

Response to 35 USC §112 rejections:

2. I have read and understood the Final Rejection mailed November 15, 2006, and the prior art that has been cited by the patent examiner of the United States Patent and Trademark Office against the subject patent application.

3. The Examiner has rejected Claims 40-46 as being indefinite for failing to meet the written description requirement. The Examiner states: "The claims lack adequate support in the specification. The claims are drawn to treating "disturbance of the membrane bound thiol:; "disturbance of intracellular thiol"; "disturbance of the thiol status" and "thiol deficient T-cells."

4. The claims 40-46 have specific support in the specification. The dosages in Claims 40, 41, 43 are described on page 8 of the specification. Claim 40 has further support with Fig. 2. Claim 41 is supported by Fig. 1. Claim 42 is supported by line 15, page 3 thru line 3, page 4 and the first paragraph under the "Summary of the Invention" subheading on page 5 and lines 5-8, page 8. Claim 43 has direct support from Fig. 3. Claim 44-46 are taken directly from line 24, page 8 thru line 3, page 9.

5. The Examiner then rejects claims 40-46 as being indefinite "because the specification does not reasonably provide enablement for using the instant composition [sic] for any "disturbance of the membrane bound thiol"; "disturbance of intracellular thiol"; "disturbance of the thiol status" and diseases arising from "thiol deficient T-cells." The specification does not enable any person skilled in the art to which it pertains, or with which it is most nearly connected, to make and use the invention commensurate in scope with the claims."

6. The claims in question do in fact enable one skilled in the art to use the instant composition by providing dosage information and experimental support for the use of the invention commensurate in scope with the claims (see statement 4 above). The term "*any* disturbance of the membrane bound thiol" is incorrectly used by the Examiner, because the claims to not use the word *any*. The term "diseases arising from "thiol deficient T-cells" is also incorrectly used by the Examiner because a person skilled in the art would accept without question that thiol defieciency in T-cells actually *causes* a disease.

7. Further, the Examiner states "While thiol deficiency has been found in many diseases there is no evidence in literature or the instant specification that improvement of thiol levels leads to a cure."

8. A disruption in the thiol-disulfide status is not considered by those skilled in the art to be a disease on its own merits. Rather it is a negative symptom of several known diseases, described on pages 1-5 of the specification of the current application. Correcting a deficiency of the thiol-disulfide, which the invention claims to do, is alleviating a known symptom of a disease, not curing it, which many successful medications already in use do. The language of the claims is directed to such a use (alleviating a known symptom of a disease) and fully enable one skilled in the art to use the invention.

9. The Examiner also states: "Clearly, not all diseases arising from thiol disturbances are known today. The specification fails to disclose how a patient, whose thiol level is low and needs treatment, can be identified without undue experimentation."

10. The argument the Examiner uses would be similar to saying not all diseases arising from bacterial infections are known today, so therefore any invention claiming use of antibiotics to treat bacterial infections is not enabled. The specification clearly outlines on pages 1-5 of the current application diseases where there *are* known disturbances of the thiol-disulfide status, and would not require any further experimentation on the patient other than diagnosing one of those diseases. Therefore, the Examiner is incorrect in his rejections based on enablement.

Response to 35 USC §103 rejections:

11. The Examiner rejects claims 40-46 as being obvious in view of Jablonka and Biewenga.

12.. The Examiner appears to have equated the treating of a disturbance of thiol-disulfide status with the correcting of glutathione (GSH) deficiency, yet the two medical conditions are not identical. Further, the prior art relied on by the Examiner is primarily directed to glutathione instead of total thiol content. Additionally, it appears that the Examiner fails to recognize and understand the synergistic effect disclosed in the application when α-lipoic acid is used in combination with ambroxol. The remainder of this declaration will address:

- Differences between thiol-disulfide status and GSH deficiency;
- Failure of the cited prior art to disclose the problem of influencing the protein-bound thiol content of cells or tissues; and
- Unexpected, synergistic (super-additive) effects of the present invention. To support the super-additive effect, additional experimental data showing the super-additive effect of the present invention will also be presented.

Thiol-Disulfide Status vs. GSH Deficiency

13. The present application is directed to a method of treating the thiol-disulfide status in cells, particulary the peripheral immune cells of a patient with diabetes mellitus. The thiol status in cells and tissue is defined as the sum of protein-bound and non-protein-bound thiolgroups. (See pages 1200-1202 in Schafer and Buettner, Free Radical Biol. Med. 2001, 30:1191-1212). Thus, the **total thiol content** of a cell is the sum of free thiols and protein-bound thiols. Protein-bound thiols are mainly present in the form of proteins containing sulphydryl groups (-SH groups). The protein-bound thiols are detected as intracellular proteins as well as membrane-bound proteins at the cell surface.

14. A typical example of a non-protein-bound species is glutathione, which counts for about 80% of the free or non-protein-bound thiol-groups detectable in a cell. However, the predominant fraction of thiol-groups is represented by protein-bound thiol-groups, especially by the amino acid cysteine. These proteins are the main component in cellular development and differentiation processes, as well as in detoxification processes. Reduced glutathione (GSH), reaches an intracellular concentration between 2-10 mM, whereas the total concentration of protein-bound thiol groups is between 15-25 mM, as observed in erythrocytes (Rossi et al., Biochim. Biophys. Acta, 1995, 1243:230-238, page 230, column 1). Thus, the content of free (non-protein-bound) thiol groups adds up to a total concentration of 10 mM, whereas protein-bound thiol groups are present in the cells up to a concentration of 25 mM. Thus, there is an excess of protein-bound thiol groups in a cell.

15. As previously mentioned, the physiological content of free thiols in cells and tissue is defined as the sum of protein-bound and non-protein-bound thiol groups (see pages 1200-1202 in Schafer and Buettner, Free Radical Biol. Med. 2001, 30: 1191-1212). On page 1200, column 2 the authors describe the concentration of protein-bound groups in cells and tissues as being much greater than that of reduced glutathione. They further state on page 1202, column 2, that the protein-bound SH groups can play a role in the antioxidant network of cells and thereby influence the redox-environment of the cell.

16. Further evidence of the limited influence or contribution of GSH on the total thiol status of a cell can be drawn from the fact that the thiol content of a cell is only reduced about 20% after selective inhibition of the GSH synthesis (Tager et al., FRBM, 29, 1160-1165, 2000). Measurements of the total thiol content in cell samples were conducted before and after the addition of buthionin sulfoximin (BSO) a known inhibitor of the glutathione synthesis. Table 2, column 2, of the Tager et al. reference shows the thiol expression indices as calculated by the ratio of thiol content of BSO-treated (GSH depleted) and untreated samples. Depending on the measuring method that is used, it can be clearly seen that the inhibition of glutathione synthesis only reduced the total thiol expression to a rather small extent.

17. Experimentally, it is possible to determine the total amount of free thiol-groups in a cell or even the amount of a specific thiol-group-containing compound, e.g. glutathione. In the present invention, however, the **total** amount **of free protein-bound and non-protein-bound** thiol-groups was determined.

18. One embodiment of the present invention treats the disturbed thiol status of a cell or tissue caused by a disturbance of the total thiol content (i.e., a disturbance of the thiol-disulfide status) by co-administration of α-lipoic acid and ambroxol. Figures 1 and 2 of the pending application show specifically the influence of a combination of α-lipoic acid and ambroxol, on the intracellular thiol status and the membrane-bound thiol status, respectively.

19. In summary, it is my opinion that a disturbance of the thiol-disulfide status is not the same as a GSH deficiency and, as discussed in the next section, that one of skill in the art would not have been motivated to combine the cited references to arrive at the claimed invention.

Prior Art Is Directed To Glutathione Only and Does Not Suggest the Claimed Invention

20. Jablonka et al.(1992) relates to the anti-oxidative and anti-inflammatory properties of ambroxol on lung tissue and alveolar macrophages in dogs. Jablonka et al. **does not indicate an** effect of **ambroxol on the total amount of free protein-bound and non-protein-bound thiol groups.** It merely suggests that an influence of ambroxol on the GSH-metabolism is a possibility. On page 63 in the last paragraph of the discussion, the authors of the study state: "...on the basis of the methodology used it is impossible to say whether Ambroxol stimulates the antioxidant enzyme production or presents the properties of the radical scavenger." Moreover, by treating the animals with ambroxol, they reached glutathione concentrations in the nanomolar range. In view of the mentioned millimolar concentration of GSH in cells and tissues this result represents a more than 100,000 times lower level, that could not have any significant biological effect.

21. Biewenga et al. (1997) refers to the antioxidant activity of α-lipoic acid and and its reduced form DHLA. The research described here includes reports of direct antioxidant activity by α-lipoic acid and influence on GSH production metabolism. Biewenga et al does not discuss the influence of α-lipoic acid on the **total** thiol-disulfide status. In fact, Biewenga et al. makes no attempt to measure thiol-didulfide status at all.

22. It would not be obvious to combine Jablonka et al.(1992) and Biewenga et al. (1997) to achieve the present invention. Jablonka et al. only teaches a general function of ambroxol as anti-oxidative agent, whereas Biewenga et al. describes the effect of α-lipoic acid on antioxidative properties of cells and also GSH synthesis. None of the references mentions the importance of

22 (cont.) the complete thiol level in a cell characterized by the sum of all protein-bound and non-protein-bound thiol groups for a dysfunction of a cell or a tissue. Furthermore, none of the references provides information about how to increase the total amount of free thiol groups in a cell or tissue. Therefore, a person skilled in the art would have had no motivation to combine the references in order to come to the beneficial solution of the claimed invention. Furthermore, no reference suggests that a synergistic effect can be had by combining, e.g., α-lipoic acid and ambroxol in a medicament.

23. Moreover, even if Jablonka et al.(1992) and Biewenga et al. (1997) were combined, a person skilled in the art would not come to the beneficial solution of a method of treatment according to pending claims 40-46. Neither reference teaches or suggests a medicament or method for the treatment of a disturbed cellular thiol-disulfide status in diabetes mellitus therapy by increasing the total **protein-bound and non-protein-bound** thiol amount in a cell according to the pending claims.

24. In summary, it is my opinion that:

- No document teaches the influence of any of the claimed compounds on the protein-bound thiol groups;
- No document teaches the application of the combined drug preparations of the present invention;
- No document teaches the synergistic, super-additive effect of the combined drug preparations of the present invention; and
- The skilled person would not have been motivated to combine the references as suggested by the U.S. patent examiner, and even if so combined, the references would not suggest the present invention.

Super-Additive Effect

25. The Examiner states that there is no clear demonstration of a synergistic effect from these (provided) results. In my opinion, the Examiner is in error because he did not take into account the effect shown for the Thiol-deficient control group when calculating the effectiveness of combining the compounds.

For example the super-additive effect is clearly demonstrated in case of membrane-bound thiol expression of lymphocytes after 48 hours treatment in Figure 2. After 2 days the singular administration of a-lipoic acid caused a 10% increase of the thio expression, whereas the singular administration of ambroxol caused 5% increase compared to the thiol-deficient control. When the two compounds were administrated in combination, the effect was NOT 15% (as to be

25 (cont.) expected). Rather, the effect of the combination shows a 38% increase in membrane thiol expression. This difference is a remarkable, super-additive effect. Similar calculations can be drawn from the intracellular thiol expression data at day 2 treatment in healthy subjects as well as in diabetic patients. This super-additive effect is striking unexpected and cannot be predicted from the prior art.

Additional Data Demonstrating the Super-Additive Effect

Influence on the Cellular Thiol Status of Monocytes in Mice

26. The influence of the combination of both drugs, a -lipoic acid and ambroxol, was investigated *in vivo*. Balb-c mice were put into a state of thiol deficiency by keeping them on a strict, thiol-free diet. The induction of thiol deficiency was controlled by the determination of the intracellular thiol content at the individual cell level monocytes. This was carried out using 5-chloromethylfluorescein diacetate in flow cytofluorimetry. Thiol deficiency was demonstrated after 10 days on the specific diet. The artificially thiol-depleted mice were treated with α-lipoic acid, ambroxol, and the combination of both drugs. Untreated thiol-deficient and healthy animals were used as controls. The drugs were administered orally in the drinking water over a length of time of 52 days. For each study group the investigations were repeated 5 times with 5 animals (n=25). In the appended Figure 8, the results of the estimated intracellular thiol content of monocytes from peripheral blood are shown. The data are given as the ratio of the mean cellular fluorescence intensity for treated or untreated thiol deficient animals to the parallel estimated healthy animals in accordance with following equation:

$$TEI \text{ (thiol expression index)} = mfi_{test} / mfi_{healthy} \times 100. \text{ (mfi: mean fluorescence intensity)}$$

27. The treatment of thiol-deficient mice with α-lipoic acid or ambroxol alone had no significant effect on the intracellular thiol content of monocytes (Fig. 8A,B). Under the treatment of the combination of α-lipoic acid and ambroxol, beginning after 14 days, a significant improvement of intracellular thiol content occurred (Fig. 8C). This effect was significant and super-additive. A maximum in the super-additive action was achieved after treatment period of 28 days, which was constant during the remaining course of the experiment. It was particularly obvious that the administration of the individual substances did not show a significant influence at any point during treatment period.

29. I declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code, and that such willful false statements may jeopardize tile validity of the application or any patent issued thereon.

Executed this 21st day of May 2007

By: /Michael Tiger, PhD/